

Outbreaks of Disease Suspected of Being Due to Human Monkeypox Virus Infection in the Democratic Republic of Congo in 2001

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Seven outbreaks of disease characterized by a pustular rash and suspected to have been caused by human monkeypox virus were investigated. The outbreaks occurred between February and August 2001 in the province of Equateur in the Democratic Republic of Congo. The outbreaks involved a total of 31 persons and caused five deaths. Specimens from 14 patients were available and were analyzed by electron microscopy, virus isolation, and PCR assays specific for monkeypox virus and varicella-zoster virus. We provide evidence that two outbreaks were indeed caused by monkeypox virus (16 cases, with four deaths), that in two outbreaks both monkeypox and varicella-zoster virus were involved (seven cases, with one death), and that two outbreaks were cases of chickenpox caused by infection with varicella-zoster virus (six cases, with no deaths). In one outbreak, no evidence for either monkeypox or chickenpox was found (two cases, with no deaths).

Monkeypox virus (MPXV) is an orthopoxvirus which can cause a smallpox-like disease in humans. The disease is endemic in the rainforests of central and western Africa. Animal antibody surveys in the Democratic Republic of Congo (DRC) suggested that squirrels and monkeys play a role in the life cycle of the virus (6). From 1981 to 1986, intensive studies were carried out in the DRC under World Health Organization (WHO) aegis. These studies identified 338 cases of human monkeypox and 33 deaths (case fatality rate of 9.8%). The majority of patients were young children (86%), and contact with animals (72%) seemed to be the major cause of infection. The secondary attack rate among family members not vaccinated against smallpox was 9.3% (6).

In 1996, Médecins sans Frontières (Doctors without Borders) reported an outbreak of suspected human monkeypox in populations living in Katako-Kombe, Kasai Oriental Province (8). Several surveys were conducted by WHO in Katako-Kombe to study the largest outbreak of monkeypox ever recorded (2, 4). The first investigation, covering February 1996 to February 1997, identified 88 cases, with a case fatality rate of 3.7% (5). Detailed epidemiological studies demonstrated a similar secondary attack rate for nonvaccinated family members (8.3%), as noted previously.

Sequence analyses targeting the hemagglutinin gene of different MPXV isolates suggested that this sequence has not changed over the years. Combined with epidemiological data, it is assumed that MPXV has a low potential for person-to-person transmission and that infection could not sustain itself in the human population (5). However, WHO and health authorities were concerned, as a significantly higher rate of hu-

man-to-human transmission (73%) was observed, compared to 28% in the 1980s WHO studies, and therefore promoted continuous surveillance of the disease.

In this report we describe the investigation of seven outbreaks of suspected human monkeypox affecting a total of 31 persons which occurred during February to August 2001 in the province of Equateur in DRC.

MATERIALS AND METHODS

Patients and specimens. Data for seven outbreaks which occurred in seven villages of the province of Equateur are summarized in Table 1 with regard to date, location, and number of persons involved. Physicians of the Yakoma hospital investigated the patient of outbreak 1; the remaining 13 patients (outbreaks 2 to 7) were investigated in the Pimu hospital by physicians of Médecins sans Frontières. Either crusted scabs or vesicle fluid samples, collected on cotton swabs, were obtained from the hospitalized patients.

Laboratory investigations. Preparation of specimens and cultivations were performed in a biosafety level IV laboratory. Swabs were soaked in 1 ml of cell culture medium (minimal essential medium [MEM]) supplemented with antibiotics and shaken vigorously. Crusted scabs were homogenized with the FastPrep™ System (Q-biogene). Briefly, crusts were placed into 2-ml screw-cap tubes containing different amounts of ceramic medium; 0.9 ml of MEM was added, and the tubes were shaken vigorously for 20 s in the FastPrep instrument. After a brief centrifugation step (1 min at 1,000 × g), the supernatants of homogenized scabs or soaked swabs were used for further analyses.

For electron microscopy, 5-μl aliquots were placed on poly-L-lysine-covered grids, stained with phosphotungstic acid (2% in distilled water), and examined on a Zeiss M10 JR electron microscope. For virus isolation, 400 μl of supernatant was inoculated onto a monolayer of African green monkey kidney cell line MA104. After 1 h at 37°C, the inoculum was removed, and the cells were washed once with 5 ml of MEM before addition of 5 ml of MEM containing 1% fetal calf serum. Cells were monitored daily for cytopathogenic effects.

Upon the appearance of plaques, the causative agent was identified as MPXV by PCR. Total DNA was directly isolated from 300 μl of supernatant of either homogenized crusts, vesicle fluid, or infected cell culture material by proteinase K-sodium dodecyl sulfate incubation, phenol-chloroform extraction, and ethanol precipitation. An orthopoxvirus-specific (3) and an MPXV-specific (9) PCR were performed as recently described. The specificity of the amplicons was determined by sequencing or by *Bgl*II restriction enzyme cleavage. In order to amplify varicella-zoster virus (VZV) DNA, the VZV LC PCR kit (Artus, Hamburg,

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TABLE 1. Suspected monkeypox outbreaks, Equateur Province, DRC

Outbreak no.	Village	Health zone	No. of cases (no. of deaths)		Patients		Onset of disease	Symptoms ^a				Electron microscopy result	MPXV isolated	PCR result	
			Suspected	Investigated	Age (yr)	Sex		Fever	Enlarged lymph nodes	Mouth	Palm & sole	Face	Trunk	Orthopoxvirus	MPXV
1	Abuzi	Yakoma	7 (1)	1	1.5	M	February	U	U	Yes	Yes	Yes	Poxvirus	+	Yes
2	Boso Mane	Pimu	6	3	18	M	February 25	No	U	U	Some	Some	Negative	-	No
					12	M	March 1	No	U	U	Some	Some	Poxvirus	+	Yes
3	Nzelao	Pimu	2	1	3	M	March 1	No	U	U	Some	Some	Negative	-	No
4 ^b	Boso Yale	Pimu	2	1	2	F	March 13	No	U	U	No	Yes	Negative	-	No
5	En Dongale	Pimu	1 (1)	1	11	M	March 10	Yes	U	U	+++	+	Negative	-	No
6 ^b	Massike Forest	Pimu	4	3	10	?	March 19	Yes	Cervical	U	+++	+++	Poxvirus	+	Yes
					10	?	March 15	No	U	U	No	Yes	Herpesvirus	-	No
					1	?	March	No	U	U	++	No	Negative	-	No
					9	?	March 8	No	U	U	No	+	Negative	-	No
					30	M	August 9	Yes	Cervical	Yes	+++	+++	Poxvirus	+	Yes
7 ^c	Liyefe	Pimu	9 (3)	4	14	M	August 8	No	Cervical inguinal	No	+	+	Poxvirus	+	Yes
					9	F	August 9	Yes	Cervical	Yes	+	+	Negative	+	No
					8	M	August 8	Yes	Cervical	Yes	No	+	Negative	+	No

^a U, unknown. Severity is indicated on a scale from + to + + + +.^b All patients belonged to the same family.^c All patients lived in the same household.^d Also enlarged inguinal lymph nodes.

Germany) was used on a LightCycler (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's instructions.

RESULTS

Clinic and epidemiology. Details of the clinical investigations and epidemiological data are presented in Table 1. Starting in early February 2001 in Abuzi village, outbreak 1 involved a total of seven persons. Specimens were taken from crusts of a 1-year-old child who had also lesions in the mouth. Outbreak 2 included six cases belonging to different families, and specimens collected from three patients were available. Outbreaks 3 and 4 involved two patients each, and specimens were collected from one of the two cases. A patient (outbreak 5) who presented with numerous lesions all over the body and who had enlarged cervical lymph nodes died the day after clinical investigation. Outbreak 6 occurred among four children of one family, and specimens were taken from three of them. The most recent outbreak (outbreak 7) occurred in August 2001 among members (one adult and eight infants) of the same household and caused three deaths.

Onset of clinical symptoms was remarkably similar for the nine cases (on 7, 8, and 9 August, respectively), suggesting a common source of infection. This source might have been a monkey found dead in the forest in the last week of July that was handled and eaten by the family (a total of 20 people were exposed to this monkey). One girl (4.5 years old) suffering from fever, shivering, enlarged cervical lymph nodes, and generalized eruptions died on 13 August; a second girl (3.5 years) with the same clinical signs died a few hours after her admission to the hospital in Pimu on 14 August; a third child, a boy 3.5 years old, died on 23 August with clinical features of generalized eruptions, conjunctivitis, pharyngitis, and pulmonary failure. The main symptoms of five patients clinically investigated on 17 August consisted of enlarged lymph nodes and pustular lesions on the palms, soles, face, trunk, and arms.

Laboratory findings. The laboratory findings of specimens obtained from 14 suspected monkeypox patients are summarized in Table 1. Typical brick-shaped orthopoxvirus particles were seen by electron microscopy in five specimens, whereas herpesvirus capsids were seen in one specimen. Inoculation of MA104 cell cultures revealed plaques characteristic of MPXV for four specimens. Formation of plaques was observed during 24 h following inoculation, and the causative agent was subsequently identified as MPXV by PCR.

Two PCR assays were used to specifically amplify either orthopoxvirus-specific or MPXV-specific sequences. In both assays, amplicons were obtained for the same seven specimens. The sequences of the amplicons obtained in the orthopoxvirus-specific assay corresponded exactly to a sequence deposited in GenBank which was obtained from an MPXV strain isolated in DRC in 1977. *Bgl*III cleavage of the 601-bp amplicon obtained in the MPXV-specific assay revealed three fragments, as has been described for 19 MPXV strains (9). VZV DNA-specific sequences were identified in six specimens.

DISCUSSION

In this report we describe findings for seven outbreaks of suspected human monkeypox. Two outbreaks were proved to

be due to MPXV (16 cases, with four deaths), while MPXV and VZV were shown to be cocirculating or coinfecting in two others (seven cases, with one death). Two outbreaks were identified as chickenpox (six cases, with no deaths). We were unable to demonstrate evidence of infection with either MPXV or VZV in outbreak 4.

In outbreak 7, the death of three patients underlines the life-threatening potential of MPXV, showing in a case fatality rate of 33%. As has been described in previous studies (6), young children (aged 3 to 5 years) were the principal victims. The sudden onset of the disease in this outbreak points to a common source of infection, most probably the dead monkey. In contrast to the crusted scabs taken during outbreaks 1 to 6, vesicle fluids collected on cotton swabs were sampled in outbreak 7. Although all swabs were positive for MPXV DNA, orthopoxvirus particles were seen only in two samples, and virus isolation succeeded only in one of those. This lower sensitivity is in contrast to the results obtained by investigation of crusted scabs. A lower sensitivity by electron microscopy and failure to isolate virus might be due to the fact that processing of viral material absorbed by swabs leads to a higher dilution of virus compared to processing crusted scabs.

We demonstrate that one patient with a fatal outcome had a dual infection with both MPXV and VZV (outbreak 5). It is the second time that a coinfection with MPXV and VZV has been reported. Hutin et al. (5) described six active cases of MPXV in one household and demonstrated serologic evidence of a recent VZV infection in five of those patients. VZV DNA was amplified from the skin lesions of two patients. It might be speculated whether dual infections are merely accidental due to simultaneous circulation of both viruses, but a mutual influence in the pathogenesis of either virus may also play a role. Further studies are needed to clarify the role of VZV in monkeypox epidemiology. Our findings in outbreaks 2, 3, and 6, i.e., amplification of VZV DNA, confirm recent reports that chickenpox can be clinically mistaken for monkeypox (7). This might partially be explained by a relative lack of expertise of health care workers.

The study of the monkeypox cases described here shows that (i) human monkeypox, although sporadic, is still a life-threatening disease in DRC; (ii) based on previous studies and the

outbreaks described here, there is no evidence that MPXV can sustain itself in the human population; (iii) handling or eating dead monkeys is one source of infection for humans; and (iv) cocirculation and/or coinfection with VZV is responsible for a number of cases associated with febrile pustular rash. The prolonged outbreak in Kasai Oriental (1, 4) that lasted from February 1996 until 1998 and the continuous reports of monkeypox cases in the DRC since 1998 give evidence that monkeypox is still a public health problem in this country. Thus, WHO will continue to support comprehensive and laboratory-based investigations to better understand the true extent of outbreaks, the epidemiology, and the clinical features of the disease.

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